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(54) Title: TRANSFORMING GROWTH FACTOR-BETA

(57) Abstract

A polypeptide transforming growth factor found in porcine platelets, having activity in the TGF-β assay and a molecular weight of about 25 kDa. The factor is a homodimer, each chain having an N-terminal sequence strikingly different from human platelet TGF-β. The factor is purified using gel filtration and reverse phase HLPC.

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TRANSFORMING GROWTH FACTOR-BETA

TECHNICAL FIELD

This invention relates to growth factors, and in particular to transforming growth factor-beta (TGF-ß), a multi-functional peptide involved in con- trolling proliferation, differentiation, and other functions in many cell types.

BACKGROUND ART

Type ß transforming growth factor (TGF-ß) is a multi-functional, hormonally active polypeptide that is synthesized by many cell types. Virtually all cells have receptors for TGF-ß. See, generally, M.B. Sporn, et al., Transforming Growth Factor-ß: Biological Function and Chemical Structure, Science, Vol. 233, p. 532-534 (1986); J. Massague, The Transforming Growth Factors, Trends in Biochem. Sci., Vol. 10, p. 239-240 (1985a). Though TGF-ß was first identified by its ability to cause phenotypic transformation of rat fibroblasts, it is now recognized as having regulatory actions in a wide variety of both normal and neoplastic cells. TGF-ß influences the rate of proliferation of many cell types, acting as a growth inhibitor and also controlling processes of adipogenesis, myogenesis, chondrogenesis, osteogenesis, epithelial cell differentiation and immune cell function. Increased expression of fibronectin, type I collagen and probably other extracellular matrix compoments is a widespread early response of cells to TGF-ß. Altera-

tions in the architecture of the extracellular matrix induced by TGF-ß could be involved in regulating the expression of specific phenotypes by this factor, while certain effects of TGF-ß on cell proliferation may be secondary to elevated expression of mitogenically active polypeptides.

Three structurally distinct cell surface glycoproteins have been identified that specifically bind TGF-ß with affinity constants in the picomolar range. J. Massague, The Transforming Growth Factors, Trends in Biochem. Sci., Vol. 10, p. 239-40 (1985). Since many cell lines display all three types of putative TGF-ß receptors, it is possible that this family of TGF-ß receptors might interact with a family of TGF-ß-related polypeptides in a situation similar to that which exists among the receptors for other families of hormonally active agents.

To date, TGF-ß, a 25 kDa protein, has been thought to be present in mammals in a single form, a homodimer of two 12.5 kDa chains linked by disulfide bonds. But cf. EPO application 85304848.6, "Polypeptide Cartilage-Inducing Factors Found in Bone" (Inventor: S. Seyedin et al.) (describes two forms of cartilage-inducing factor, CIF-A and CIF-B, each of which is a homodimer, the two forms having different amino acid sequences) with S. Seyedin et al., "Cartilage-inducing Factor-A: Apparent Identity to Transforming Growth Factor-ß," J. of Biol. Chem. Vol. 261, p. 5693-95 (1986).

DISCLOSURE OF INVENTION

The invention relates to a unique form of TGF-ß which has been found in porcine platelets. The factor, designated TGF-ß2, is a homodimer having an approximate molecular weight of 25,000 daltons. Each strand of the dimer has a partial N-terminal amino

acid sequence strikingly different from human TGF-ß.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is an elution curve plotting absorbance at 230nm and 240nm against elution of TGF-ß on a acetonitrile gradient;

Figure 2 shows the silver stain from SDS-polyacrylamide gel electrophoresis of TGF-ß forms 1, 2 and 3;

Figures 3a-3c are plots of partial amino acid sequences of TGF-ß forms 1, 2, and 3, respectively.

Figure 4 shows autoradiography of electrophoresed, affinity-labeled TGF-ß receptors in a differential competition for human TGF-ß1, porcine TGF-ß1, and porcine TGF-ß2;

Figure 5 shows autoradiography of electrophoresed, affinity labeled TGF-ß receptors using the chloramine T and Bolter-Hunter methods;

Figure 6 shows the silver stain from SDS-polyacrylamide gel electrophoresis of human TGF-ß, porcine TGF-ß1 and porcine TGF-ß2 under reducing and non-reducing conditions; and.

Figures 7a-7c are elution curves similar to Figure 1, comparing human TGF-ß with porcine TGF-ß1.

BEST MODE FOR CARRYING OUT THE INVENTION

Initial extraction of TGF-ß from platelets is accomplished by lysing the cells and then centrifuging to remove insoluble material. The supernatant is then precipitated, e.g., with ethanol-ether, resuspended, and fractionated by gel-filtration, such as over a Bio-Gel P-60 column. Further purification may be accomplished by reverse phase HPLC, preferably on successive C-4 and C-18 columns.

Subsequent fractionation on a Synchropak C-4 column resolves the TGF-ß into three peaks at approximately 32%, 34% and 36% acetonitrile (see Fig. 1).

The least hydrophobic peak corresponds to TGF-ß1, a homodimer having an N-terminal amino acid sequence, so far as is known, identical to human TGF-ß. The most hydrophobic peak corresponds to TGF-ß2, a distinct homodimeric form of TGF-ß having an N-terminal amino acid sequence substantially different from TGF-ß1. The middle peak corresponds to TGF-ß3, the heterodimer of a single strand each of forms 1 and 2. Each of the forms has an approximate molecular weight of 25 kilodaltons, and has approximately equivalent activity in the TGF-ß assay, i.e., promotes approximately equivalent growth of unanchored NRK cells in semisolid medium.

In previous studies, three cell surface glycoproteins of 280-330 kDa, 85-95 kDa and 65 kDa, respectively, have been identified which have high affinity receptors for human TGF-ß1. J. Massague, Subunit Structure of a High Affinity Receptor for Type ß Transforming Growth Factor. Evidence for a Disulfide-linked Glycosylated Receptor Complex, J. Biol. Chem., Vol. 260, p. 7059-66 (1985). The 280-330 kDa receptor species is a component of a larger (600 kDa) receptor complex held together by disulfide bonds. The ability of these three putative TGF-ß receptor structures to interact with TGF-ß1 and TGF-ß2 was examined by affinity labeling with ^{125}I-TGF-ß1 cell lines that were known to display on their surface all three receptor types. The 280 kDa receptor form displays high affinity for both TGF-ß1 and TGF-ß2. Occupancy of this receptor type by TGF-ß1 or TGF-ß2 correlates with their ability to inhibit cell proliferation. In contrast, cell surface receptors of 65 kDa and 85 kDa that have high affinity for TGF-ß1 display lower affinity for TGF-ß2. The existence of distinct forms of TGF-ß that interact differently with

TGF-ß receptors could provide flexibility to the regulation of tissue growth and differentiation by the TGF-ß system.

Purification of TGF-ß from porcine platelets.

Fresh porcine blood was obtained from slaughterhouses, and the platelets were extracted by adding a solution of 1% Triton X-100, 0.2 M EGTA in a ratio of 1 ml/g, freezing to -20°C and thawing five times and centrifuging at 5-10 xg for 30 minutes to remove insoluble material. Supernatant from the centrifugation was mixed with 4 parts of acidic ethanol, 50 parts of 95% ethanol, 14 parts distilled water, 1 part concentrated HCl and adjusted to pH 5.2 with concentrated ammonium hydroxide, in accordance with the procedure of A.B. Roberts, et al., Transforming Growth Factors: Isolation of Polypeptides from Virally and Chemically Transformed Cells by Acid/Ethanol Extraction, Proc. Natl. Acad. Sci. USA, Vol. 77, p. 3494-98 (1980).

The proteins were precipitated with two volumes of cold anhydrous ethanol and four volumes of cold anhydrous ether and allowed to stand for about 20 minutes. Precipitate was collected by centrifugation or rapid filtration through Whatman No. 1 paper and resuspended in 1 M acetic acid (about 3-4 ml per gram of tissue). Insoluable matter was removed by centrifugation at about 5-10 xg for 10-30 minutes, and the supernatant was then concentrated, as by lyophilization with resuspension in 1M acetic acid.

This suspension was then fractionated over successive Bio-Gel P-60 gel filtration columns (100-200 mesh) in the absence of and then in the presence of urea as described by R.K. Assoian, et al., Transforming Growth Factor-ß in Human Platelets: Identification of a Major Storage Site, Purification and

Characterization, J. Biol. Chem., Vol. 258, p. 7155-7160 (1983). The first elution was done on a 5.0 cm x 100 cm column at a flow rate of about 40 ml/h, the column having been equilibrated in 1 M acetic acid. 10 ml fractions were collected and those fractions having TGF-ß activity (as described below) were pooled and concentrated by lyophilization for further purification.

Active fractions from the first column were dissolved in 5 ml of 1 M acetic acid containing 8 M ultra-pure urea and gel-filtered on the second Bio-Gel P-60 column (5 cm x 80 cm) at a flow rate of about 20 ml/hour, the column equilibrated with the sample solvent. Fractions of 10 ml were collected. (To preclude the formation of cyanate in the solvent, the ultra-pure urea may be dissolved at pH 2 in 1 M acetic acid, the resulting solution being adjusted to final conditions by addition of glacial acetic acid and water.) Aliquots of selected column fractions were again tested for TGF-ß activity (as described below). Fractions containing the peak of TGF-ß activity were pooled and concentrated, e.g., by pressure filtration through an Amicon YM5 membrane.

The pooled TGF-ß fractions were further purified at ambient temperature on two successive reverse phase HPLC columns. In the first column, a Synchropak C-4 column (10 mm x 250 mm), a linear gradient of 15-30% n-propanol in H_2O/0.1% trifluoroacetic acid was used at a flow rate of 1 ml/min, the gradient changing at 0.1%/min. TGF-ß eluted at approximately 22% propanol. Fractions having TGF-ß activity were pooled and diluted 1:1 with 1M acetic acid to reduce the propanol concentration, and then loaded on the second column, a Synchropak C-18 (10 mm x 250 mm). A linear gradient of 20-30% n-propanol in H_2O/0.1%

trifluoroacetic acid was used on this column at a flow rate of 1 ml/min, the gradient changing at 0.05%/min. TGF-ß eluted as a broad peak beginning at approximately 24% n-propanol.

TGF-ß from the second HPLC step was again chromatographed over a Synchropak C-4 column (10 mm x 250 mm), eluted with a linear gradient of 25-40% acetonitrile in H_2O/0.1% trifluoroacetic acid at a flow rate of 1 ml/min, the gradient changing at 0.1%/min. Figure 1 shows the 230nm and 240nm absorbance elution profiles of fractions containing TGF-ß. Three distinct protein peaks appear at approximately 32%, 34% and 36% acetonitrile. The least hydrophobic peak is designated TGF-ß1, the most hydrophobic TGF-ß2, and the middle peak TGF-ß3.

To confirm the existence of these three distinct peaks, forms 1, 2 and 3 from three different runs were pooled separately. Samples (about 10 ug of protein) from each of these pools were mixed and rechromatographed over the Synchropak C-4 column as described above. Three separate peaks of absorbing material were again produced. Aliquots of fractions across the profile of absorbing material were subjected to SDS-polyacrylamide gel electrophoresis and visualized by silver staining, shown in Figure 2. The arrow identifies the 25 kDa band present in fractions from all three peaks. Positions of 29 kDa and 18 kDa molecular weight markers are also indicated.

The above procedures yield substantially purified quantities of TGF-ß typically in the range of about 2 ug per gram of platelets, representing an approximately 500,000 fold purification. Purity of 95-97% as determined by silver staining of samples run on non-reducing SDS gels, amino acid composition analysis, and N-terminal sequencing is reproducibly

achievable.

In quantification of total TGF-ß recovered from the above purification, form 1 represented the predominant form (approximately 65-80%) of TGF-ß present. The amounts of form 2 and form 3 varied from preparation to preparation with form 3 typically present in a lesser amount (generally about 5-10% of total TGF-ß recovered). Form 2 was present generally in the range of 15-25%. Quantification may be accomplished by integrating the peaks of the elution curves. Approximate quantification may also be ascertained by cutting out the elution peaks and weighing the cut paper.

Comparison to Human TGF-ß.

Human platelet TGF-ß prepared and analyzed by the above procedures did not yield the three peaks found for porcine TGF-ß. Figures 7a-7c respectively show elution peaks of 10 ug porcine TGF-ß1 (Fig. 7a), 10 ug human TGF-ß (Fig. 7b), and a mixture of 5 ug pTGF-ß1 and 5 ug hTGF-ß (Fig. 7c). The single peak of human TGF-ß comigrated with porcine TGF-ß1, the least hydrophobic of the porcine platelet TGF-ß peaks.

All forms of TGF-ß from porcine platelets exhibited the characteristic disulfide-linked dimeric structure when they were visualized by silver-staining SDS-polyacrylamide electrophoresis gels. See Figure 6. Samples containing 50 ng of human TGF-ß (a, d), 50 ng of porcine TGF-ß form 1 (b, e), 125 ng of porcine TGF-ß form 2 (c, f), or 100 ng of human TGF-ß (g) were electrophoresed on 15% (acrylamide:Bis, 20:1) polyacrylamide SDS gels in the presence and absence of reductant, in accordance with U.K. Laemmli, Cleavage of Structural Proteins During Assembly of the Head of Bacteriophage T4, Nature, Vol. 227, p. 680-85 (1970). The reduced samples a-c were run in the presence of 50

mM dithiothreitol. The positions and molecular weights of protein standards also are indicated.

TGF-ß Activity Assay.

Material in each of the three peaks was evaluated in the so-called TGF-ß assay to determine activity. This assay determines the ability of the polypeptide to induce anchorage-independent growth in non-neoplastic NRK fibroblasts by measuring the formation of colonies in soft agar.

The test material was sterilized by lyophilization of 1 M acetic acid solutions in sterile tubes. The residue was then redissolved in binding buffer at 10 times the final concentration used in the assay and centrifuged to clarity. Samples to be tested were suspended in 0.3% agar (Difco, Noble agar) in Dulbecco's modified Eagle medium (GIBCO) supplemented with 10% calf serum (GIBCO) penicillin (100 units/ml), streptomycin (100 ug/ml) and 5 ng/ml of EGF. A portion (0.7 ml containing 3500 cells of the resultant mixture) was pipetted onto a 0.7 ml base layer (0.5% agar in the supplemented medium) in each of three 35-mm petri dishes. Plates were then incubated at 37°C for 7 days in a humidified 10% CO_2 atmosphere without further feeding.

The assay may be read unfixed and unstained at 1 week. Alternately, the plates may be stained with 0.7 ml of a sterile solution of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (0.5 mg/ml in water) layered over the agar and incubated for 24 hours. After removal of excess dye solution, the plates may be scored in a bright-field microscope (with or without projection onto a screen), counting the number of colonies in a unit field.

TGF-ß activity is defined as the effective dose (ED) resulting in 50% of maximal colony formation

(colony size greater than 3000 um^2) in the presence of EGF (2.5 ng/ml). The maximal response of the assay is about 2500 colonies. Each of the three TGF-ß forms was approximately equivalent in activity in this assay, having an ED_{50} of about 0.1-0.4 ng/ml (ED_{50} being the effective dose yielding 50% of maximal response).

Amino Acid Sequence Analysis of Porcine TGF-ß

Nonreduced samples of 25 kDa TGF-ß1 (180 pmol), TGF-ß2 (130 pmol) and TGF-ß3 (400 pmol) were subjected to N-terminal automated Edman amino acid degradation in the presence of polybrene using an Applied Biosystems Model 470A gas-phase sequenator. Phenylthiohydantoin (PTH) amino acid derivatives were quantitated with a Hewlett-Packard 3390A integrator. Yields are shown in Figures 3a-c, corresponding to TGF-ß forms 1-3, respectively. The amino acid identified in each cycle is indicated. The open symbols in Figure 3c indicate the yield of PTH amino acid corresponding to the amino acid residue listed in the bottom row of the deduced sequence. Figure 3 shows only the first 43 residues of TGF-ß3; additional studies have identified the first 50 residues of the amino acid protein. (Since human TGF-ß has 112 amino acids and has a molecular weight of 25 kDa, and since pTGF-ß3 is a heterodimer of forms 1 and 2, forms 2 and 3 are believed to also have 112 amino acids.)

The sequence of the N-terminal 50 amino acids of porcine TGF-ß1 was found to be identical to the N-terminal sequence of human TGF-ß, as follows:

```
                  5                   10
Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-Ser-Thr-Glu-Lys-Asn-
15                  20                  25
Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-
    30                  35                  40
Gly-Trp-Lys-Trp-Ile-His-Glu-Pro-Lys-Gly-Tyr-His-Ala-Asn-
        45                  50
Phe-Cys-Leu-Gly-Pro-Cys-Pro-Tyr.
```

In contrast, analysis of TGF-ß2 yielded an N-terminal amino acid sequence that was strikingly different from the sequence of TGF-ß1:

```
                  5                   10
Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Glu-Asp-Asn-
15                  20                  25
Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-
    30                  35                  40
Gly-Trp-Lys-Trp-Ile-His-Glu-Cys-Cys-Gly-Tyr-Asn-Ala-Asn-
        45                  50
Phe-Cys-Ala-Gly-Gly-Cys-Pro-Tyr.
```

N-terminal amino acid sequencing of TGF-ß3 yielded a mixed sequence identical to the combined sequences of TGF-ß1 and TGF-ß2. Those cycles in form 3 corresponding to residues in which TGF-ß1 and TGF-ß2 differed yielded an approximately equimolar amount of both amino acid derivatives. All other cycles yielded a single amino acid derivative corresponding to the residue shared by TGF-ß1 and TGF-ß2 in that position, indicating that TGF-ß3 corresponds to the heterodimer consisting of one chain of TGF-ß1 linked to one chain of TGF-ß2.

Receptor Interactions.

Two representative cell lines, Mv1Lu and BRL, were affinity-labeled with 10 pM human ^{125}I-TGF-ß1 in the presence of incremental picomolar concentra-

tions of native human TGF-ß1, porcine TGF-ß1 or porcine TGF-ß2. Triton extracts of cells (one tissue culture well per lane each) were electrophoresed, and the labeled receptors were displayed by autoradiography, as shown in Figure 4. The labeling inhibition patterns obtained indicated that porcine TGF-ß2 has a lower affinity for the 85 kDa and 65 kDa receptors than porcine TGF-ß1. Thus, the amount of labeling still associated with the 85 kDa and 65 kDa receptors in BRL cells in the presence of 500 pM p.TGF-ß2 (about half of maximal) was similar to that remaining in the presence of 40 pM human TGF-ß1 or porcine TGF-ß1. In contrast, the 280 kDa TGF-ß receptor component displayed similar affinity for all types of TGF-ß. This incremental competition pattern was also obtained with human GM370 fibroblasts, A549 carcinoma cells and mouse 3T3-L1 fibroblasts (not illustrated). In Mv1Lu cells, the 65 kDa receptor had a slightly higher affinity for TGF-ß1 and TGF-ß2 than did the 85 kDa receptor (see Fig. 4).

Affinity labeling experiments using Mv1Lu cells and iodinated human and porcine TGF-ßs confirmed the different ability of TGF-ß1 and TGF-ß2 to interact with the 85 kDa and 65 kDa receptors. Monolayers of Mv1Lu cells were affinity-labeled with 10 pM human ^{125}I-TGF-ß1, 10 pM porcine ^{125}I-TGF-ß1, or 25 pM porcine ^{125}I-TGF-ß2, all prepared by the chloramine T method. Other monolayers were affinity labeled with 100 pM porcine ^{125}I-TGF-ß1 or 100 pM porcine ^{125}I-TGF-ß2 prepared with Bolton-Hunter reagent. Some samples were affinity-labeled in the presence of an excess of unlabeled human TGF-ß1 (+). Samples were then subjected to electrophoresis and autoradiography, the results of which are shown in Figure 5. Human and porcine TGF-ß1 iodinated by the chloramine-T yielded

similar affinity-labeled receptor patterns whereas porcine TGF-ß2 preferentially affinity-labeled the 280K receptor. Similar results were obtained with the use of TGF-ß1 and TGF-ß2 radiolabeled via their amino groups with ^{125}I-labeled Bolton-Hunter reagent. No evidence was found that the porcine TGF-ßs bound proteins other than the 280 kDa, 85 kDa and 65 kDa proteins.

Apart from the above receptor interaction studies, research has not been completed on the functions of TGF-ß2 in comparison to TGF-ß1 or TGF-ß3. All forms are active in the TGF-ß assay however, and thus it is apparent that TGF-ß2 possesses at least a portion of the molecular functions of TGF-ß. Consequently it is likely that TGF-ß2 may have utilities similar to the contemplated therapeutic utilities of TGF-ß. Such utilities include repair of tissue injury caused by trauma, burns, surgery, or debility in the aged, regulation of metabolic conditions such as osteoporosis, and use as an antiinflammatory or immunosuppressive agent, among others. See, generally, M.B. Sporn, et al., Transforming Growth Factor-ß: Biological Function and Chemical Structure, Science, Vol. 233, p. 532-34 (1986); S. Seyedin, Cartilage-Inducing Factor-A: Apparant Identity to Transforming Growth Factor-ß, J. of Biol. Chem., Vol. 261, p. 5693-95 (1986).

CLAIMS

What is claimed is:

1. A polypeptide growth factor characterized in that the factor:

a. is found in porcine platelets;

b. has activity in the TGF-ß assay; and

c. is a homodimer having an approximate molecular weight of 25,000 daltons.

2. The factor of Claim 1 wherein each the chain of the dimer has the following N-terminal sequence:

```
                5                   10
Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Glu-Asp-Asn-
15                  20                  25
Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-
    30                  35                  40
Gly-Trp-Lys-Trp-Ile-His-Glu-Cys-Cys-Gly-Tyr-Asn-Ala-Asn-
        45                  50
Phe-Cys-Ala-Gly-Gly-Cys-Pro-Tyr.
```

3. A substantially purified polypeptide growth factor characterized in that the factor is found in porcine platelets, has activity in the TGF-ß assay, and is a homodimer having an approximate molecular weight of 25,000 daltons, each chain of the dimer having the N-terminal amino acid sequence:

```
                5                   10
Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Glu-Asp-Asn-
15                  20                  25
Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-
    30                  35                  40
Gly-Trp-Lys-Trp-Ile-His-Glu-Cys-Cys-Gly-Tyr-Asn-Ala-Asn-
        45                  50
Phe-Cys-Ala-Gly-Gly-Cys-Pro-Tyr.
```

4. A composition comprising substantially pure porcine TGF-ß wherein the growth factor of Claim 1 is present in an amount of at least about 15%.

Fig.1

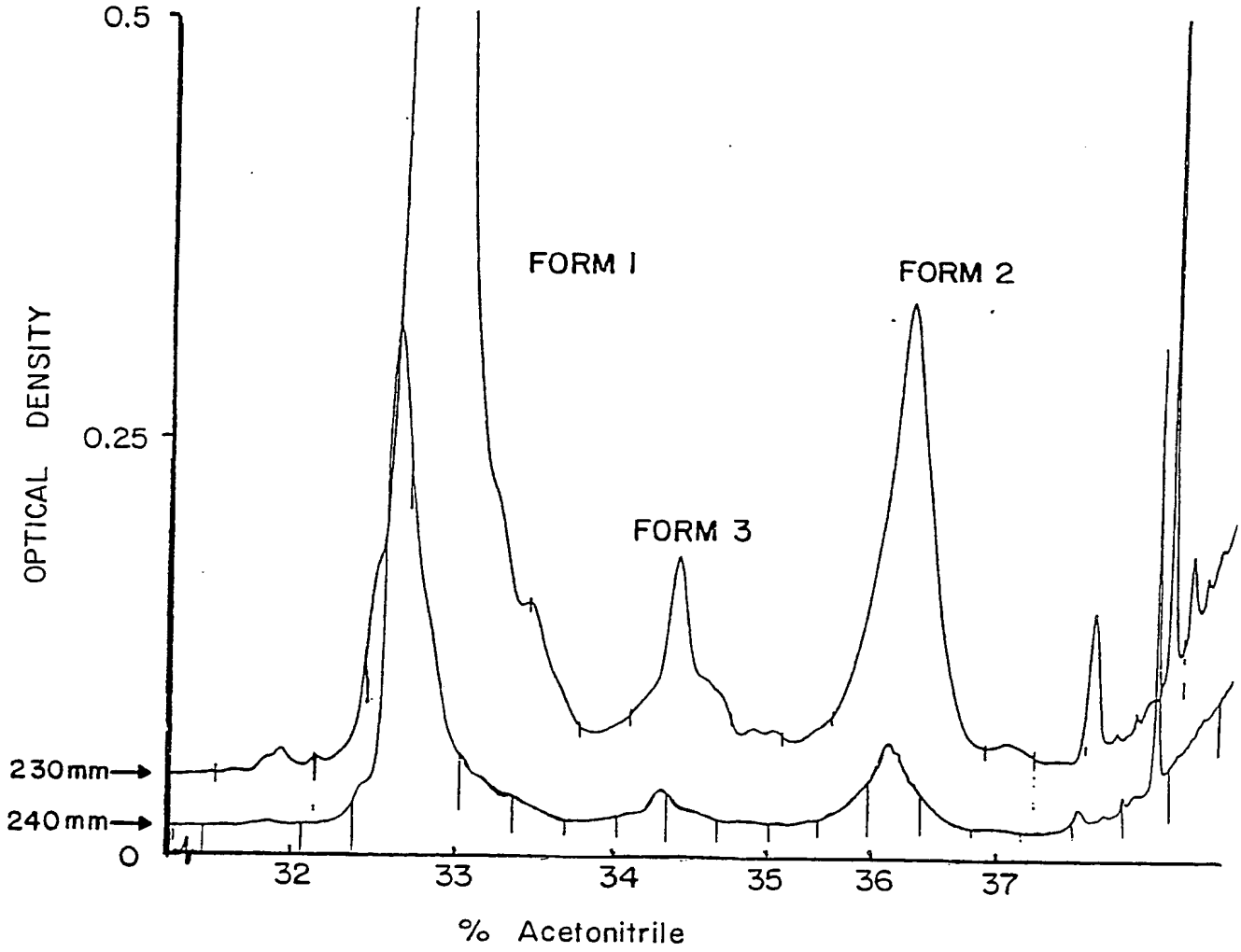


Fig. 2

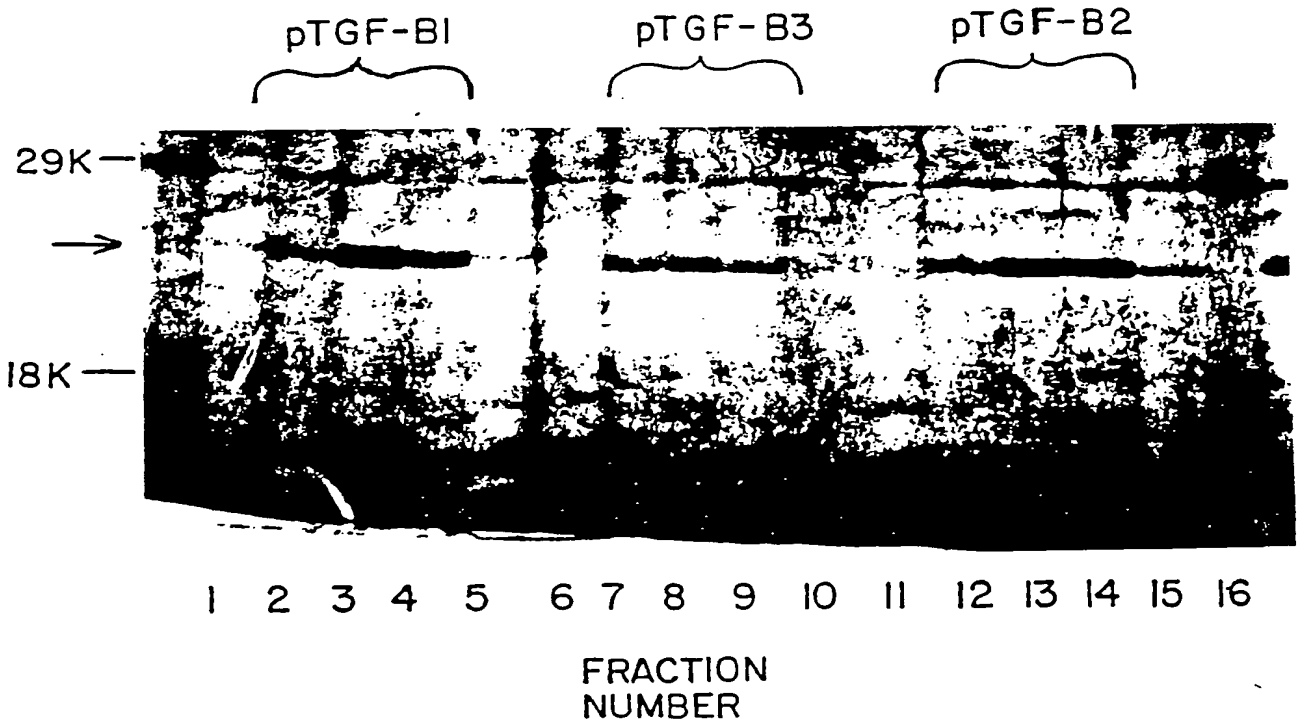

pTGF-B1
pTGF-B3
pTGF-B2
29K
18K
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
FRACTION NUMBER

Fig. 3A

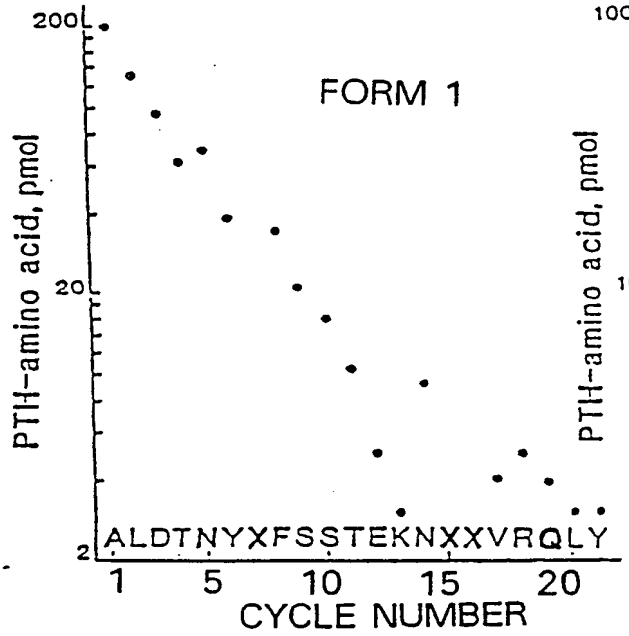


Fig. 3B

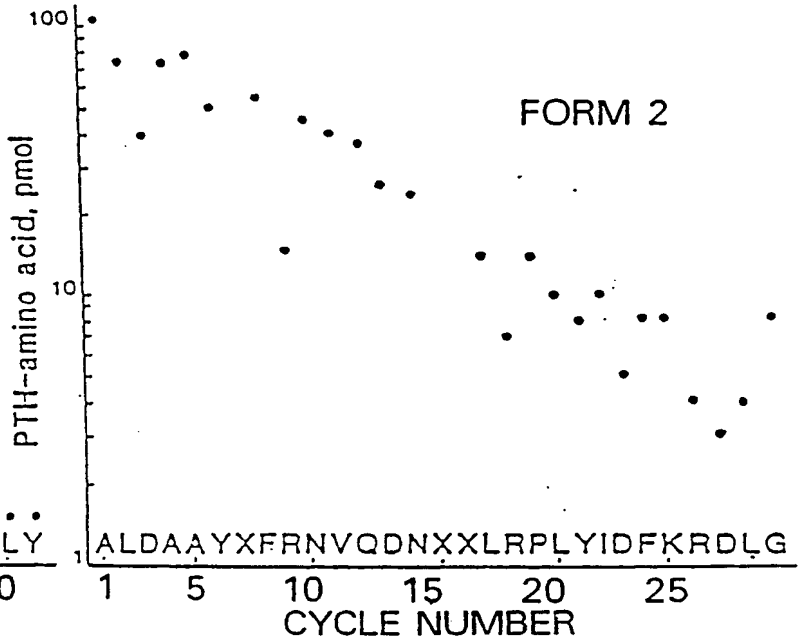


Fig. 3C

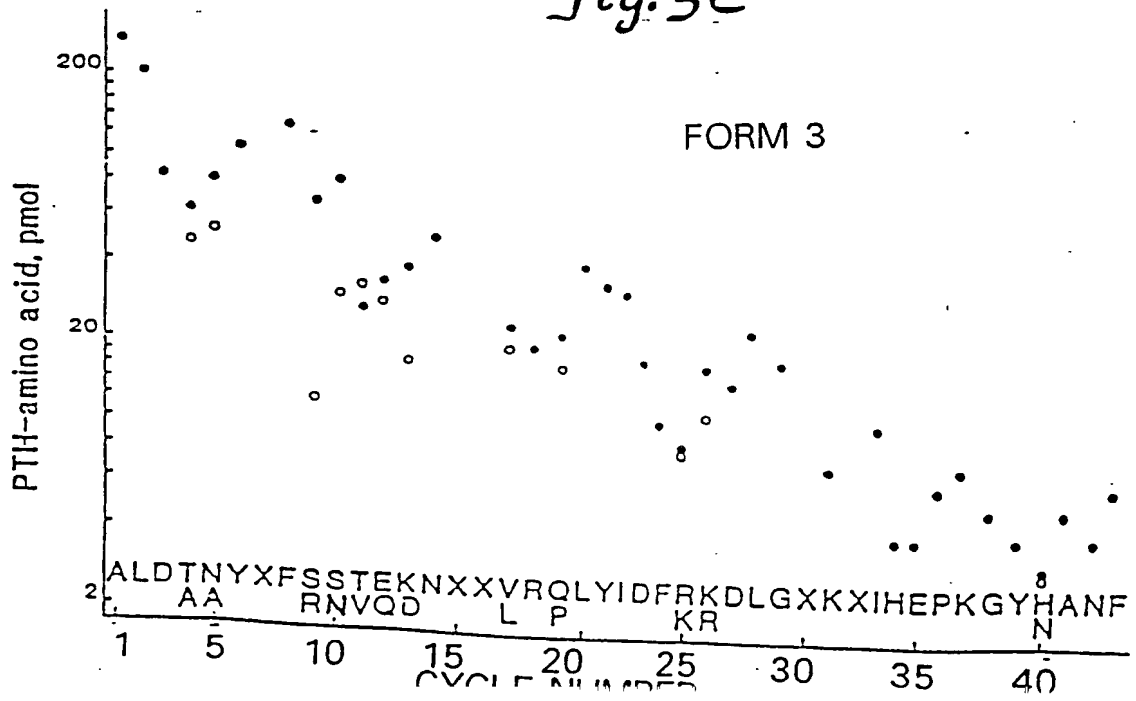


Fig. 4

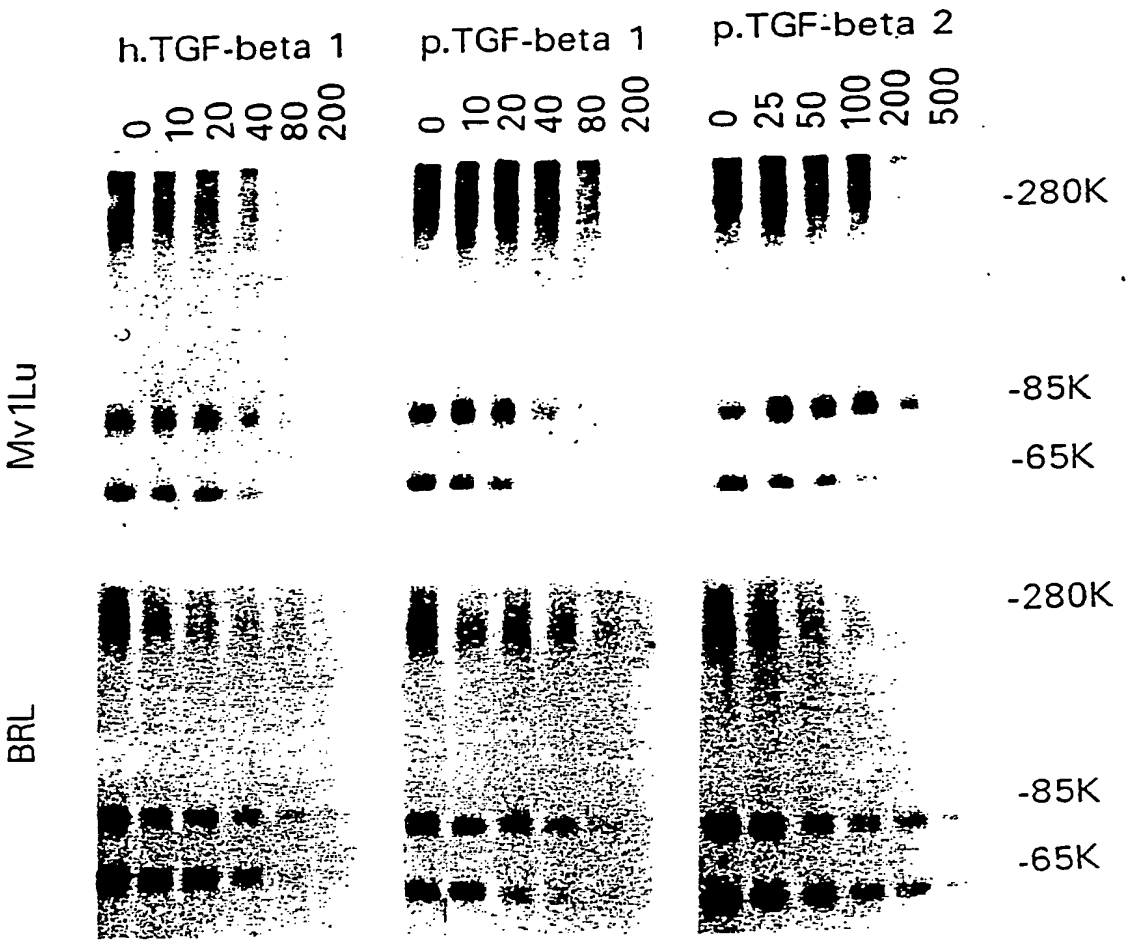


Fig.5

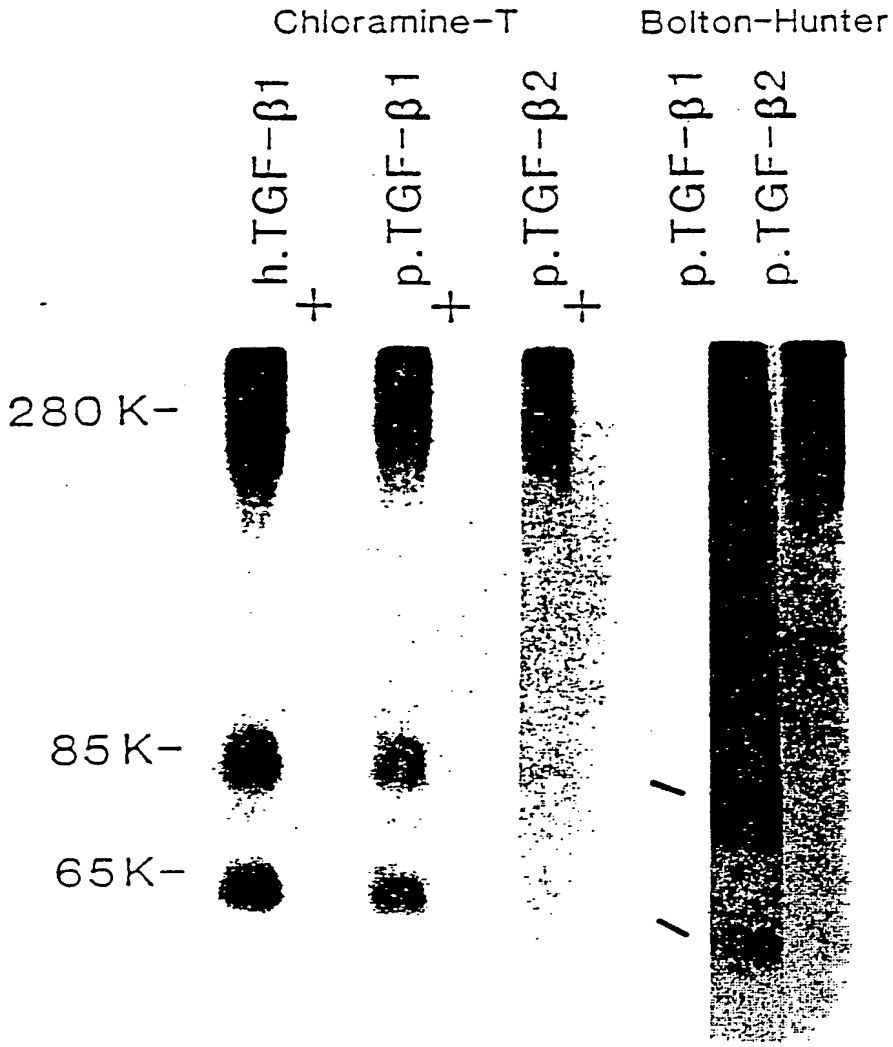


Fig. 6

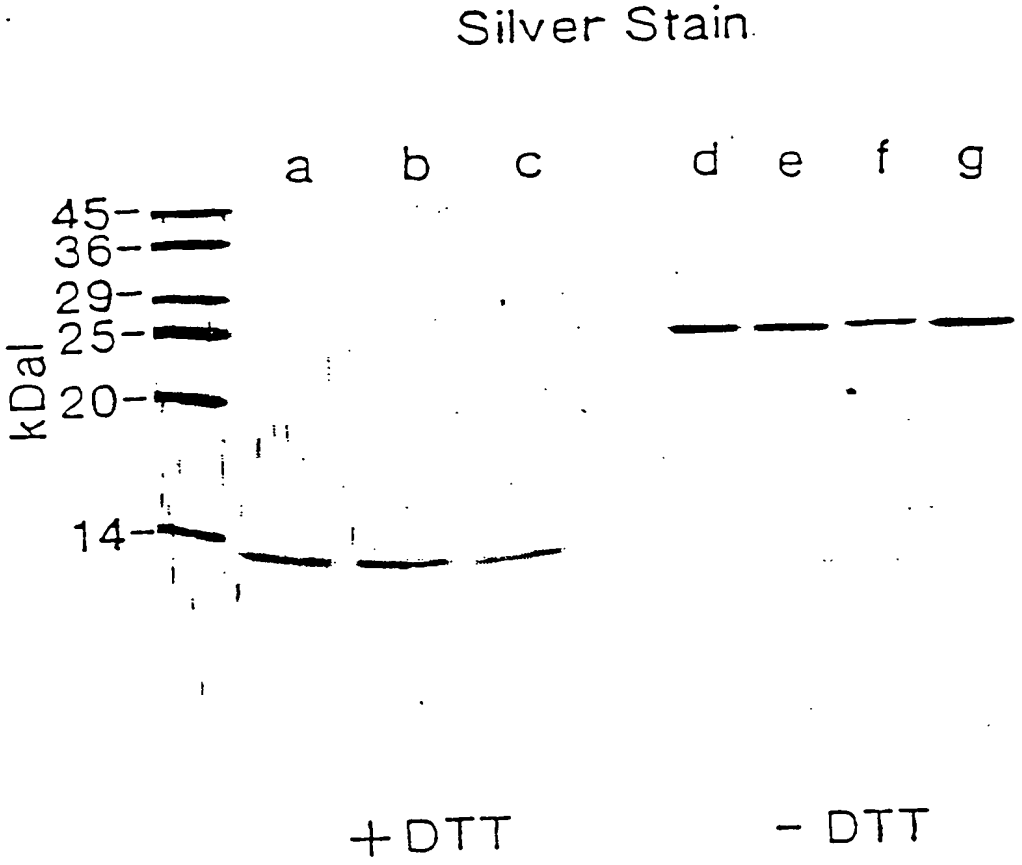


Fig. 7A

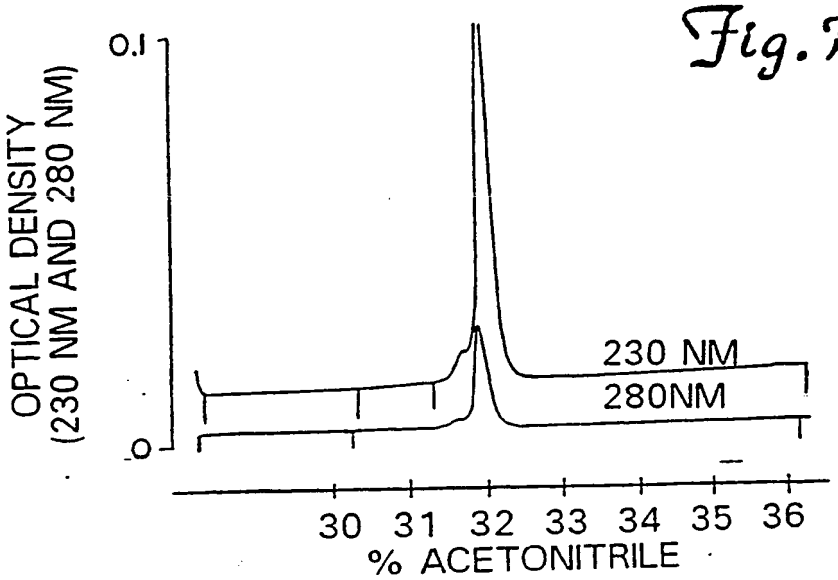


Fig. 7B

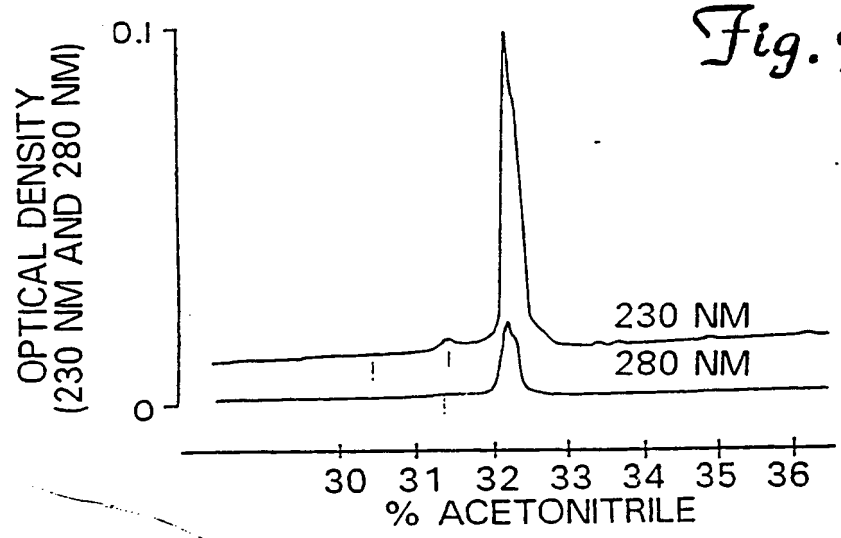
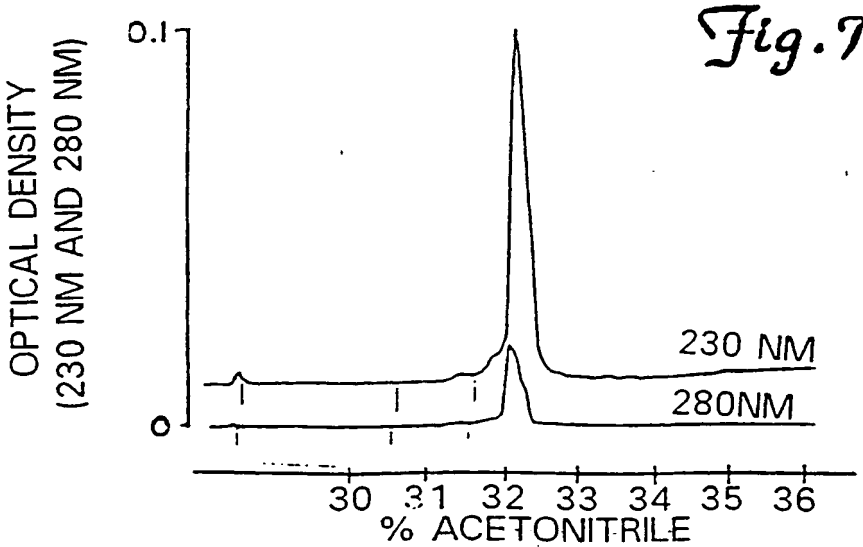


Fig. 7C



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00270

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL[4] C07K 15/00; A61K 37/02, 37/24
US CL. 530/399, 350, 300, 380; 514/2, 12, 21

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U. S.	530/399, 350, 300, 380, 827, 829; 514/21, 12, 21

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	PCT, A, WO/84/01106, (SPORN), MARCH 1984.	1-4
X	BIOCHEMISTRY, VOL. 22, ISSUED 1983, PURIFICATION AND PROPERTIES OF TYPE β TRANSFORMING GROWTH FACTOR FROM BOVINE KIDNEY", (ROBERTS), PAGES 5692-98.	1-4
X	JOURNAL BIOLOGICAL CHEMISTRY, VOL. 261, ISSUED APRIL 1986, "THE MURINE TRANSFORMING GROWTH FACTOR-β-PRECURSOR," (DERYNCK), PAGES 4377-79.	1-4
Y	JOURNAL BIOLOGICAL CHEMISTRY, VOL. 256, ISSUED JUNE 1983, "TRANSFORMING GROWTH FACTOR-β-IN HUMAN PLATELETS," (ASSOIAN), PAGES 7155-60.	1-4

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 MARCH 1988	06 MAY 1988
International Searching Authority	Signature of Authorized Officer
ISA/US	Garnette D. Draper GARNETTE D. DRAPER

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEE)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	TRENDS IN BIOCHEMICAL SCIENCE, ISSUED JUNE 1985, "THE TRANSFORMING GROWTH FACTOR," (MASSAGUE), pg. 237-240, SEE PG. 238-39.	1-4
Y	SCIENCE, VOL. 233, ISSUED AUGUST 1986, "TRANSFORMING GROWTH FACTOR-β :BIOLOGICAL FUNCTION AND CHEMICAL STRUCTURE," (SPORN), PG. 532-34, SEE PG. 533.	1-4
Y	JOURNAL BIOLOGICAL CHEMISTRY, VOL. 261, ISSUED MAY 1986, "CARTILAGE-INDUCING FACTOR A: APPARENT IDENTITY TO TRANSFORMING GROWTH FACTOR-β," (SEYEDIN), PG. 5693, SEE PG. 5694.	1-4
Y	EP, A, 0169,016, (SEYEDIN), JANUARY 1986	1-4
Y	EP, A, 0,200,341, (DERNYNCK) DECEMBER 1986.	1-4